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Histopathological changes in snail, *Pomacea canaliculata*, exposed to sub-lethal copper sulfate concentrations

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ABSTRACT

The acute toxicity test of Cu including range-finding and definitive test, was performed on golden apple snails, *Pomacea canaliculata*. The median lethal concentrations (LC_{50}) of Cu at exposure times of 24, 48, 72 and 96 h were 330, 223, 177 and 146 μg/L, respectively. *P. canaliculata* were exposed to Cu at 146 μg/L for 96 h to study bioaccumulation and histopathological alterations in various organs. Snails accumulated elevated levels of Cu in gill, and lesser amounts in the digestive tract, muscle, and digestive gland. Histopathological investigation revealed several alterations in the epithelia of gill, digestive tract (esophagus, intestine, rectum), and digestive gland. The most striking changes were observed in the epithelium of the gill in which there was loss of cilia, an increase in number of mucus cells, and degeneration of columnar cells. Similar changes occurred in digestive tract epithelium. The digestive gland showed moderate alterations, vacuolization and degeneration of cells and an increase in the number of basophilic cells. We concluded that, *P. canaliculata* has a great potential as a bioindicator for Cu, and a biomarker for monitoring Cu contamination in aquatic environment.

1. Introduction

Heavy metals encompass a wide range of substances that are non-degradable, highly persistent in the environment and which may be incorporated into the tissues of living organisms (Ortíz et al., 1999). Among heavy metals, Cu plays various physiological roles for a number of key metabolic enzymes in plants and animals (Watanabe et al., 1997). In gastropod mollusks, it is involved in hemocyanin metabolism (Gullick et al., 1981; White and Rainbow, 1985). However, increased concentration of Cu in organisms is potentially toxic since it interferes with numerous physiological processes. The major lethal effects of Cu in gastropod mollusks are disruption of the transporting surface epithelium and osmoregulation, eventually causing water accumulation in mollusk tissues (Cheng, 1979).

High levels of Cu (> 200 mg/kg) were recently reported in the sediments of tributaries feeding into Beung Boraphet, a large freshwater lake in Nakhon Sawan province, Thailand (Dummee et al., 2012). These most likely resulted from the excessive use of Cu-based fungicides, molluscicides, and pesticides in the rice paddies surrounding the tributaries (Dummee et al., 2012). Golden apple snails, *Pomacea canaliculata*, which were abundant in the area, contained high levels of Cu (81.8–127.8 mg/kg) in their visceral mass and foot.

Several tools or biomarkers may be used to manage heavy metal contamination, such as toxicity assessment, histopathological evaluation, and biochemical assessment. Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystem, and for identifying suitable organisms as bioindicators (Shuhaimi-Othman et al., 2012). In addition, acute toxicity tests can help in the detection, evaluation, and abatement of pollution by providing reliable estimates of safe concentrations of toxicants (Ahsanullah et al., 1981). Histopathological alterations reveal effects of pollution on the tissues of exposed organisms, and provide early indications of toxicity (Sawasdee et al., 2011).

Mollusks have long been regarded as promising bioindicator and biomonitoring subjects because they are abundant, highly tolerant to many pollutants, and exhibit high accumulation of heavy metals (Lau et al., 1998; Shuhaimi-Othman et al., 2012). Several studies reported on the acute and chronic toxicity of heavy metals, and attendant histopathological alterations in gastropod mollusks, such as *Filopaludina martensi martensi* (Jantataeme et al.,

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1996), *Babylonia areolata* (Supanopas et al., 2005; Tanhan et al., 2005), *Melanoides tuberculata* (Shuhaimi-Othman et al., 2012), *Pomacea paludosa* (T. Hoang et al., 2008; T.C. Hoang et al., 2008), and *P. canaliculata* (Dummee et al., 2012; Kruatrachue et al., 2011). In mollusks, the digestive gland is thought to play the most important role in metabolism of endogenous and xenobiotic compounds and is likely important in the storage and regulation of metals in terrestrial and aquatic gastropods.

The introduced golden apple snail (*P. canaliculata*) is an agricultural pest in Asia and is now the most widespread snail species in Thailand where it is ubiquitous in aquatic habitats. They feed not only on rice but also on taro and lotus plants, causing much loss to the farmers. The present work aimed to assess the potential of *P. canaliculata* as a bioindicator and biomonitor of Cu in the aquatic environment. We investigated the acute toxicity in *P. canaliculata* exposed to Cu and evaluated Cu accumulation and associated histopathological manifestations in the soft tissues including the digestive organs.

2. Materials and methods

2.1. Chemical

The stock of Cu solution (1000 μg/L) was prepared by dissolving Cu sulfate (hydrated form; $CuSO_4 \cdot 5H_2O$) in deionized water according to APHA et al. (2011). Concentrations were expressed as micrograms of Cu ions (Cu^{2+}) per liter (μg/L) of solution. The stock solution was left for 1 day to equilibrate. Nominal Cu concentrations (0, 100, 150, 200, 250, 400, and 800 μg/L) were then prepared from the stock solution diluted to 1 L with deionized water in pre-acid washed aquarium.

2.2. Experimental animal

P. canaliculata eggs were collected from unpolluted natural ponds in Kanchanaburi province, Thailand. Eggs were hatched in the laboratory and juvenile snails were reared in the aerated aquaria with dechlorinated water at 25–27 °C and daily fed with lettuce until they were 2 months old. The uniform snails (shell length 2–3 cm) were used for acute toxicity tests and histopathological study.

2.3. Acute toxicity study

The static (non-renewal, without food) technique was used for the acute toxicity test. The toxicity range-finding test consisted of a down-scale (100, 10, 1 and 0.10 μg/L of Cu) abbreviated static acute test in which groups of organisms were exposed to several widely spaced sample dilutions in a logarithmic series, and a control, for a period of 96 h. The definitive test was performed to follow-up the range-finding test. During the acclimatization (24 h) snails were fasted to prevent the binding of Cu to the accumulated feces (Ng et al., 2011). No mortality of *P. canaliculata* was detected during this period.

One liter of each test solution (and controls) was added to 4 L glass aquaria at the nominal concentrations previously described. Three replicates for each concentration, including controls, were performed. Ten snails were placed in each aquarium and were not fed during the period of the bioassay. The dead and living snails were counted daily throughout the 96-h period. Dead snails were removed immediately to avoid the bacterial infection of other living snails. The percentage survival and mortality were calculated for each metal concentration. Snails were taken to be dead if there was no movement when the foot region was prodded with the metal needle or if there was no activity after 5 min of placing them in water (Otitoloju et al., 2009).

2.4. Accumulation study

P. canaliculata were exposed to the median lethal concentration (LC_{50}) for 96 h. Ten snails were placed in each of three replicates including controls. Two replicates were used to obtain the Cu accumulation analysis and the third was used for the histopathological study. After 96 h, the soft tissues were separated from shells, snail organs (gill, digestive tract and digestive gland) and head–foot (muscle) were isolated. The tissue samples were oven dried at 80 °C, and 0.5 g of each dried samples was digested with 67% conc. HNO_3 (67%) according to APHA et al. (2011). The Cu concentrations were then determined using a flame atomic absorption spectrophotometer (FAAS; SpectrAA-55B). The Cu concentrations in each digested organ were determined by comparing its absorbance with known Cu standard solutions. The method detection limit was calculated by a standard procedure which is based on the analysis of ten samples of the matrix with the analyte. The lowest detection limit for Cu was 0.5 μg/g dw.

2.5. Bioaccumulation efficiency

Bioaccumulation is the process in which a chemical substance is absorbed in an organism by all routes of exposure including dietary and ambient environment sources (Arnot and Gobas, 2006). The bioaccumulation efficiency of Cu in *P. canaliculata* was evaluated using two parameters. First, the bioaccumulation capacity (BAC) was the ratio of the concentration of the metal in snail organs after 96 h of exposure to the level detected in the same organs of control snails (Otitoloju et al., 2009). Second, the bioconcentration factor (BCF) was the ratio of the metal concentration in snails to the concentration in water (T. Hoang et al., 2008).

2.6. Histopathological study

After 96 h of exposure, the gill, digestive tract and digestive gland of *P. canaliculata* were dissected and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 for 12 h. Organs were washed with 0.1 M sodium cacodylate buffer three times before they were post-fixed in 2% osmium tetroxide (OsO_4) for overnight. Samples were washed in 0.1 M sodium cacodylate buffer, dehydrated with the graded series of acetone, and embedded in aradite resin (Thophon et al., 2003). The semi-thin sections (1 μm) were prepared on an ultramicrotome, mounted on glass slides, stained with 1% toluidine blue in 1% borax, and examined under a light microscope (Olympus CH40).

2.7. Statistical analysis

The dose–response data was analyzed by Probit analysis based on a computer program (SPSS version 15.0). The indices of toxicity measurement derived from this analysis were lethal concentrations (LC) at 25%, 50%, 75% and 99%. Analysis of variance (ANOVA) was carried out to compare the Cu accumulation in several treatments and organs mean at $P < 0.05$ level of significance (Thophon et al., 2003).

3. Results

3.1. Toxicity study

The percentage mortality of *P. canaliculata* generally increased with increasing Cu concentration and exposure time (Table 1). A Cu concentration higher than 0.40 μg/L caused very high mortality